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Cardiac Effects of Botulinal Toxin

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Abstract—Crystalline type A botulinal toxin rapidly caused temporary bradycardia and electrocardiographic (ECG) changes in mice, rats, rabbits and dogs. In addition, in the dog the force of contraction was measured and found to be depressed. The ECG changes were indicative of conduction defects. The hemagglutinin present in the toxin played no role in the effects on the heart, since a derivative toxin without hemagglutinin also caused these phenomena. The cardiac effects were spontaneously reversible in the intact animal without removal of the toxin. On the other hand, in the *in vitro* isolated heart of the rat, recovery from the cardiac effects occurred only after the toxin was washed out of the preparation. The findings are consistent with, but do not prove, a physical rather than a chemical mechanism for the effects of toxin on the heart.

Introduction

Little attention has been paid to the occurrence of cardiac effects caused by botulinal toxin. Recent reviews of botulism poisoning (Simpson, 1981; Thesleff and Lundh, 1979; Thesleff, 1981) have barely mentioned cardiac levels or do not discuss the subject at all. In clinical studies (Petty, 1965; Ciccarelli and Gimenez, 1981; Sonnabend and Sonnabend, 1981), disorders of the heart either were not reported or were not considered to be characteristic responses in human poisonings. Effects of botulinal toxin on the isolated cat heart (Rosenblum, 1966) and on the vagus innervation of the heart of the cat, dog, rabbit (Dickson and Shevky, 1923) and frog (Erzina and Mikhailov, 1956) have been reported. In this past work, no account was taken of the existence of hemagglutinin associated with the neurotoxin

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(Lamanna and Lowenthal, 1951). Thus the possibility has not been ruled out that the effects observed were due to extraneous material in the toxic culture fluid or to the hemagglutinin associated with the neurotoxin. Here we describe the cardiac effects of progenitor crystalline type A botulinal toxin containing hemagglutinin and of the hemagglutinin-free derivative neurotoxin on the mouse, rat, rabbit, and dog as observed in electrocardiographic (ECG) recordings. Described is a previously unreported spontaneous and rapidly reversible bradycardia caused by the toxin.

Methods

All observations were made on the spontaneously beating heart without the use of experimental stimulation. This differs from past work cited, in which experimental stimulation of heart preparations was employed.

Toxin

Crystalline type A toxin that had hemagglutinin as a constituent was provided by E. J. Schantz of the University of Wisconsin. G. Sakaguchi, University of Osaka Prefecture, provided a purified sample of type A toxin free of hemagglutinin (Sakaguchi *et al.*, 1981). The toxins were dissolved in a sterilized phosphate-0.2 % gelatin buffer (pH 6.2–6.7) for storage and i.v. injection; this diluent in the amounts used and without toxin did not cause any discernible physiological changes in laboratory animals. Dosages of the toxin were measured as mouse LD_{50} units.

Type A antitoxin and toxoid

These reagents were made available by the Centers for Disease Control of the U.S. Public Health Service, Atlanta, Ga.

Experimental animals

Male and female animals were used. The mice were Charles River CDl and ICR strains weighing 25–35 g, and the rats were Charles River Sprague-Dawley and Wistar strains weighing 300–500 g. The New Zealand white rabbits weighed 3–4.5 kg and the dogs, adult beagles from the Food and Drug Administration-bred colony, weighed 9–12 kg.

Physiological measurements

A Hewlett-Packard polygraph was used to record measurements of physiological responses before and after exposure to toxin. Heart rates and

ECGs were recorded under light anesthesia induced by i.p. injection of sodium pentobarbital (mouse 15 mg/kg, rat 25 mg/kg, rabbit 10 mg/kg). A few unanesthetized mice and rats were also studied and showed no difference in measurements compared with anesthetized animals. Subcutaneous electrodes were inserted in appropriate limbs for monitoring the Lead II ECG and heart rate. After readings stabilized the toxin was given i.v.

In the dog, respiration, heart rate, ECG recordings and arterial blood pressure were monitored. The polygraph used was other than the one for the smaller laboratory animals. The dogs were anesthetized with sodium pentobarbital (35 mg/kg, i.v.). Cannulas were inserted in the right femoral vein and artery for drug injection and blood pressure measurements, respectively. Electrodes were inserted in the s.c. tissue of appropriate limbs for monitoring the Lead II ECG and heart rate. Respiration was recorded from a cuffed endotracheal tube inserted in the trachea and connected to a differential pressure transducer.

Isolated heart preparation

The spontaneous heart rate was measured for rats and dogs. Vagal nerves were not preserved and no experimental stimulus, either electrical or chemical for the beat was provided for the isolated heart preparations.

For rats, a Langendorff preparation was used with 52 cm of water perfusion pressure. The beating heart was rapidly removed from the body of an anesthetized rat and placed in pH 7.2 isotonic Krebs-Henseleit solution. The aorta was tied to a plastic cannula for continuous perfusion with Krebs-Henseleit solution kept at 36° C. The solution was oxygenated with a mixture of 95 % O_2 and 5 % CO_2. The perfusate from the heart was allowed to drip continuously and was collected in a graduated cylinder for measuring flow rate. The heart rate was recorded by means of a pair of electrodes attached to the surface of the heart and connected to a Hewlett-Packard polygraph for ECG recording. The preparation was allowed to stabilize for 5 to 15 min before the toxin solution was injected into the aortic cannula in a volume of 0.2 to 0.5 ml. After exposure to the toxin, the preparation was washed out with fresh toxin-free Krebs-Henseleit solution and allowed to reequilibrate before a second exposure to toxin.

The isolated dog heart preparation has been described by Vick and Herman (1971). The heart was excised from an anesthetized dog and perfused with 500 ml of heparinized autologous blood. Heart rate, perfusion pressure, ECG and force of contraction were measured continuously. The toxin was injected directly into the inflow side of the perfusion tubing and allowed to recirculate throughout the experiment.

Results

The crystalline toxin and the hemagglutinin-free toxin had the same qualitative effects on the heart, namely bradycardia and changes in the ECG. Thus, hemagglutinin apparently does not play a role in the cardiovascular changes resulting from exposure to the toxin. On a weight to weight basis, hemagglutinin-containing toxin was less potent than the hemagglutinin-free toxin in causing cardiac changes; a larger amount was needed to produce the effects and recovery was more rapid (Tables I and II, compare where toxin doses overlap). This greater potency might be thought to be related to the smaller molecular size of the hemagglutinin-free toxin (Lamanna *et al.*, 1970). On the other hand, potency expressed as molar specific activity (number of molecules required to cause a given effect) does not indicate the crystalline toxin to be less active in causing cardiac effects than the hemagglutinin-free toxin. The crystalline toxin has a molecular weight of 900,000 and possesses 240×10^6 mouse LD_{50}/mg nitrogen while the hemagglutinin-free toxin has a molecular weight of 150,000 and possesses 500×10^6 mouse LD_{50}/mg nitrogen (Sakaguchi *et al.*, 1981). Thus the weight of crystalline toxin needed for a particular biological activity would have to be at least 3 times greater than for a hemagglutinin-free toxin before it could be said to be less potent in terms of molar specific activity. There is still another way of thinking of potency. If the assumption is made that the same number of cell receptors are tied up for the same LD_{50} of the large and small molecule toxins, then the finding (Lamanna *et al.*, 1970) that the toxic effects of the small molecule are elicited more rapidly than those of the large molecule, permits saying that the small molecule is more potent. The definition of potency elected affects the judgment of relative potency of the different molecular size toxins. It is also a fact that crystalline toxin can dissociate with separation of the hemagglutinin component from the neurotoxic component. Such a separation has been reported in the case of i.v. injection of rabbits with crystalline toxin (Hildebrand *et al.*, 1961). The extent of disassociation varies with solvent conditions so that the situation for crystalline toxin dissolved in blood and blood-free salt solutions cannot be considered to be the same. We do not know the extent and variability in separation of the hemagglutinin from the crystalline toxin under our experimental conditions, so definite conclusions about relative potency of the 2 states of the toxin are not possible.

In mice and rats individual responses varied greatly. To date dose-response curves have not been obtained because of the large number of animals that would be required. However, the data show a trend for prolongation of lag time and a quicker recovery from bradycardia with decreases in toxin doses. At doses that caused death in a few hr, complete

TABLE I

Heart rates (% of normal rate) of individual mice after i.v. injection of hemagglutinin-free botulinal toxin (1)

Time after toxin injection (min)	Toxin dosage (mouse LD_{50} units)								
	82,000	20,500	5,000	1,025	300	38	19	5	3
5	78, 80	91, 90	90, 89	94, 100	63, 83	90, 94	94, 90	100, 105	92, 83
15	70, 67	70, 61	58, 60	88, 100	53, 74	55, 60	59, 71	83, 95	100, 74
30	45, 44	56, 50	74, 50	72, 72	58, 58	44, 60	47, 100	83, 81	110, 95
45	40, 40	56, 90	105, 90	59, 40	84, 48	40, 60	47	100, 71	
60	35, 44	56			106, 81	44, 76	59	100, 92	
75	30, 40	56				106, 106	71		
90	35, 55	62					82		
105	56, 72	70							
Approximate time of death (hr)	5, 3.33	4.25, 2.5	2.8, 2.8	24	24	24	24	Alive after 5 days	Alive after 5 days

(1) Heart rate at 0 time was considered as 100 %.

TABLE II

Heart rates (% of normal rate) of individual mice injected i.v. with crystalline botulinal toxin containing hemagglutinin [1]

Time after toxin injection (min)	Toxin dosage (mouse LD_{50} units)				
	4000	400	40	20	10
5	80, 83	95, 86	100, 100	94, 91	100, 96
15	70, 72	83, 61	74, 100	77, 70	105, 80
30	70, 89	90, 61	70, 97	124, 91	100, 120
45	75, 117	90, 86	90, 113		105,
60	85	103			

[1] Heart rate at 0 time was considered as 100 %. All mice died within 12 hr of exposure to toxin.

TABLE III

Effect of botulinal toxin on ECGs

Species	P-R interval	QRS	Q-T	Type of arrhythmia
Mouse				
Intact	Prolonged [a]	Prolonged	Prolonged	Bradycardia
Rat				
Intact	Prolonged	Prolonged	Prolonged	Bradycardia
Isolated heart [b]	— [c]	—	—	Bradycardia and AV premature beat
Rabbit				
Intact	Prolonged	Prolonged	Prolonged	Bradycardia and bigeminal rhythm
Dog				
Intact [d]	—	—	S-T segment depression	Bradycardia, bigeminal rhythm, and ventricular extra systole
Isolated heart	—	—	—	Bradycardia and ventricular fibrillation

[a] Prolonged means extended in time.
[b] Flow rate decreased in isolated rat heart.
[c] Dash indicates not measured.
[d] The data recorded are effects unaccompanied by respiratory difficulties of the animal. Variable blood pressure changes, decreased contractibility, and increased perfusion pressure were observed in the isolated dog heart.

TABLE IV

Heart rate (% of normal rate) of the isolated rat heart with repeated exposure to botulinal toxin (1)

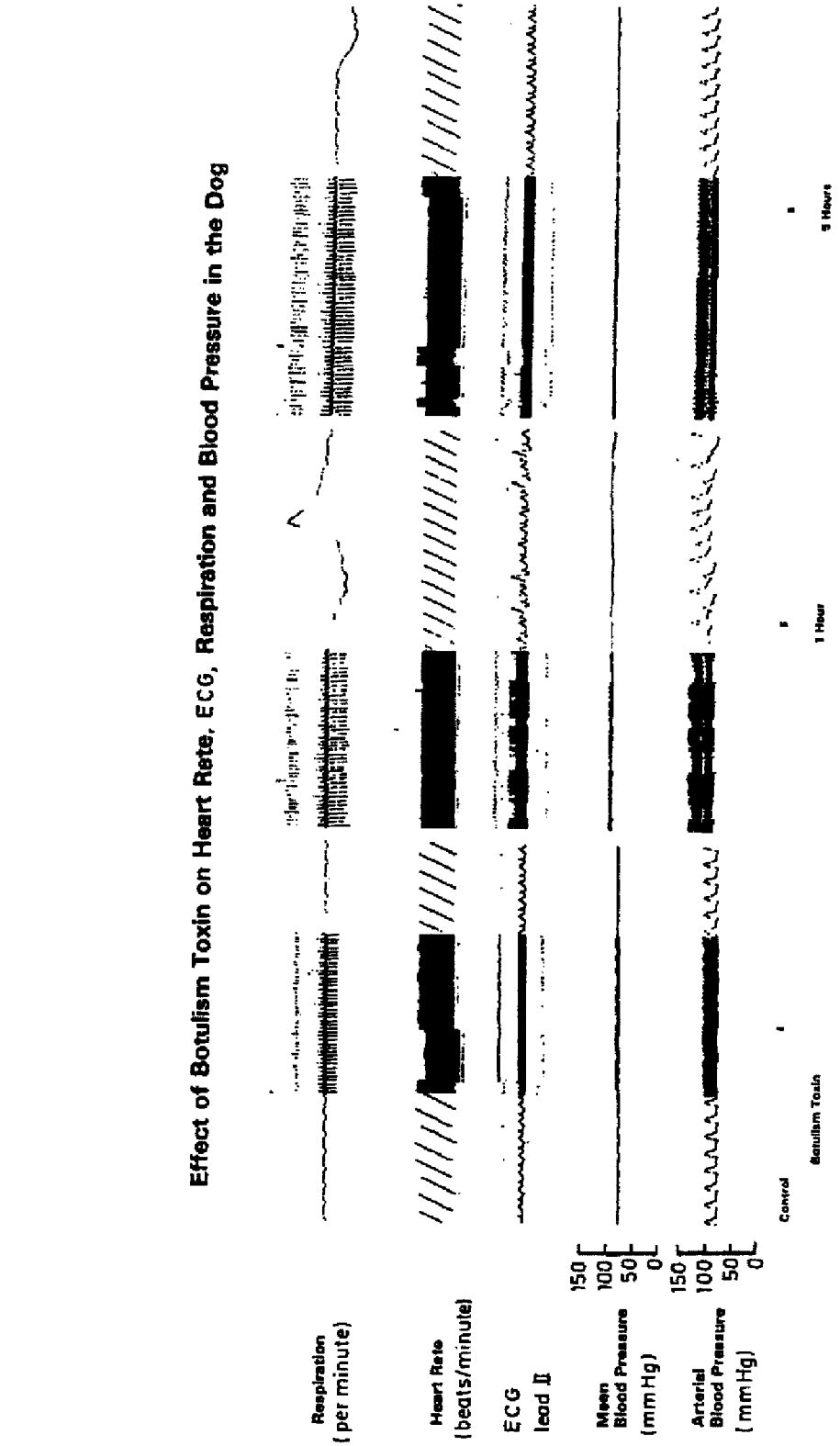
Time of successive exposure (min)	Recovery	Lowest value
Hemagglutinin-free toxin (41,000 mouse LD_{50} units)		
0	100	
0–10	90	72
10–20	96	72
20–30	96	66
30–70	96	78
70–100	84	66
100+	90	72
Hemagglutinin-containing toxin (20,000 mouse LD_{50} units)		
0	100	
0–5	108	66
5–10	78	66
10–15	90	72
15–20	90	72
20+	84	60

(1) Toxin was flushed out of the preparation within 2 min after exposure and the heart rate recovered within 10 min of the exposure. Toxin was reintroduced into the preparation at 10-min intervals.

recovery of the normal heart rate before death was often incomplete (Table I). At low doses that killed rats and mice many hr or days after injection, full cardiac recovery was reached before the animal died. Reduction of the heart rate to below 30 % of the normal rate was not observed; in the majority of animals, the heart rate was in the range of 45–90 % of the normal rate and was only roughly dependent on the dose. The deaths of animals recorded in the Tables are attributed to respiratory paralysis because of signs of respiratory muscular failure. But these signs appear many minutes or hours after the more rapidly occurring reductions in heart beats. Thus the effects of the toxin on the heart and respiratory paralysis are independent events.

Thesleff and Lundh (1979) and Thesleff (1981) showed that above a certain quantity of toxin there is no further effect on release of neurotransmitter. In other words, when the cellular receptors for the toxin have been saturated at a particular dose, increases beyond this dose are without further effect. Consistent with this is the lack of reduction in heart rate below about 30 % of normal. This probably represents the point of

Effect of Botulism Toxin on Heart Rate, ECG, Respiration and Blood Pressure in the Dog



saturation of cellular receptors by the toxin and the residual automaticity in the heart rate not subject to poisoning by the toxin.

Table III summarizes representative ECG findings for the rat and rabbit. The cardiac effects were preventable by use of specific antitoxin at doses preventing death. Toxoid was without effect when given to the rat at a dose of 0.014 mg nitrogen which is equivalent to 304×10^6 mouse LD_{50} of crystalline toxin based on a reported value of 240×10^6 mouse LD_{50}/mg nitrogen of crystalline toxin (Sakaguchi *et al.*, 1981). Slowing of the heart rate was the prominent common feature of exposure to toxin. This bradycardia had the following characteristics: in the intact rat and rabbit, 5–30 min elapsed before slowing of the heart rate occurred. The effect in the intact animal was spontaneously reversible and the disturbance could last only a short time (30–60 min); a single lethal dose could cause the cardiac effects, with death of the toxin-poisoned animals occurring hours or days after recovery of the heart rate.

In control rats injected with anesthetic and toxin-free diluent the heart rate over a 2-hr period varied over a range of 93–114 % of normal. Increased heart rate was not observed in the toxin injected rats. The heart rate for 13 anesthetized rats was 430 beats/min $\pm$ 5 standard error of the mean while the rate for 10 isolated hearts was 215 ± 11.

Isolated rat hearts showed a rapid decrease in the intrinsic heart rate and the coronary flow rate decreased to 10–40 % of normal, with recovery taking place within min after toxin was flushed out (Table IV). Continuous perfusion with toxin in the perfusion fluid maintained the decrease in intrinsic heart rate during the entire perfusion period. Toxin neutralized with antitoxin did not affect the isolated rat heart preparation.

The administration of toxin to the various species produced reproducible ECG changes indicative of conduction defects. Thus, the Lead II ECG revealed prolongation of the P-R, QRS, and Q-T intervals (Table III).

The dose of hemagglutinin-free toxin given to the dogs was about 4,000,000 mouse LD_{50} units, which is about 4000 mouse LD_{50} units per ml

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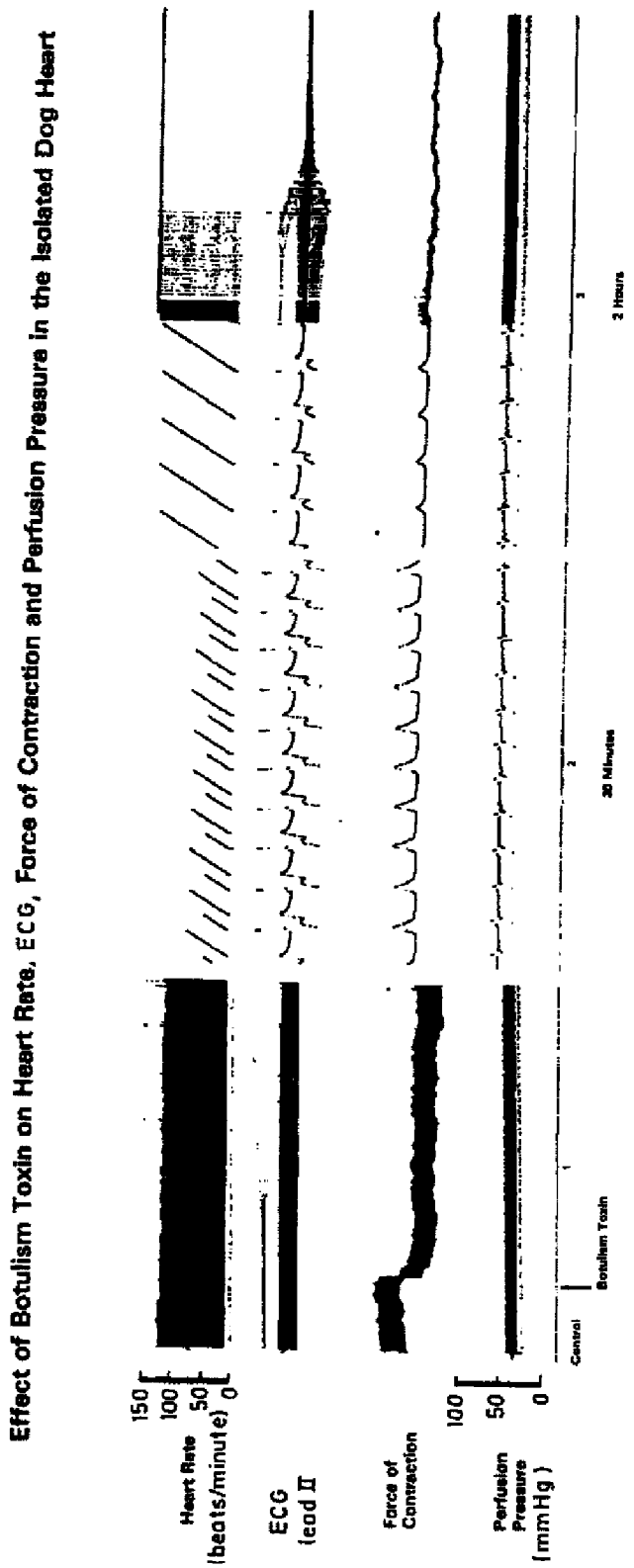
FIG. 1

Effect of crystalline hemagglutinin-containing type A toxin (8×10^6 mouse LD_{50} units) on respiration, heart rate, ECG, and blood pressure in the anesthetized adult beagle dog given toxin i.v. Note change in ECG and arterial pulse tracing 1 hr after injection of toxin and recovery at 5 hr. The toxin had no effect on arterial blood pressure or respiration during the first hr after injection; however, at time of arrhythmia, there was a bigeminal pulse trace.

Parameters measured are calibrated as follows: (1) Heart rate = beats per min, measured on a fast trace by counting each beat for 1 min; (2) ECG: intervals measured in msec, amplitude in mV; (3) Mean blood pressure = an electrical mean which averages systolic and diastolic pressure differences; (4) Arterial blood pressure = measured as systolic and diastolic pressure on a 0 to 200 mmHg scale; (5) Force of contraction = measured on a Walton-Brodie strain gauge.


Effect of Botulism Toxin on Heart Rate, ECG, Force of Contraction and Perfusion Pressure in the Isolated Dog Heart
Heart Rate (beats/minute)
150
100
50
0
ECG lead II
Force of Contraction
Perfusion Pressure (mmHg)
100
50
0
Control
Botulism Toxin
20 Minutes
2 Hours

of blood, based on an estimated blood volume of 100 ml/kg in the dog. The dose led to death in 1 to 8 days after toxin exposure.

Nine anesthetized dogs were given the toxin i.v. The injection was followed by an immediate slight decrease in blood pressure and a variable increase in heart rate. This tachycardia was not seen in the other animal species and might be a temporary response to the stress of injection or an inhibiting effect on the vagal nerve. These changes returned to beginning values within 5 to 15 min after exposure to toxin. From 30 to 60 min, 6 of the 9 dogs began to show ECG changes; these consisted of depression of the T wave and, in 2 dogs, premature ventricular contractions (extrasystole) (Fig. 1). There were no changes in respiration during the time the ECG changes were occurring. The extrasystole was accompanied by a bigeminal pulse pattern that persisted until the ECG returned to normal (Fig. 1). Complete ECG recovery including bradycardia took place within 1.5 to 3 hr after exposure to toxin. The decrease in heart rate was in the range of 35 to 135 beats per min. The normal pulse in the anesthetized dog ranged from 180 to 210 beats pr min.

Bilateral vagotomy, atropine at a level of 0.5 mg/kg and a combination of the two in dogs did not prevent arrhythmias, ECG changes, or bradycardia from occurring after the toxin injection. The results were the same when atropinized (1 mg/kg, i.p.) rats were given the toxin. At the peak of the ECG effects 2 dogs were given antitoxin sufficient to neutralize the toxin given. No alteration in cardiac response was observed except that one of these dogs survived the exposure to toxin.

The isolated perfused dog heart responded to toxin (8000 mouse LD_{50}/ml in 500 ml of perfusate) in much the same manner as did the intact animal. The heart rate of the isolated dog heart was 90–105 beats/min and for the dose of toxin used dropped to 75–90 beats/min. These changes would not be expected in a control unchallenged heart preparation (Vick and Herman, 1971). There was a persistent decrease in both the heart rate

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FIG. 2

Effect of crystalline hemagglutinin-containing type A toxin on heart rate, ECG, force of contraction and perfusion pressure of an isolated dog heart perfused with blood with toxin added. Note that at 30 min and 2 hr there was a progressive decrease in heart rate and force of contraction accompanied by a marked elevation in perfusion pressure. Following toxin, the heart rate decreased from an average of 90–105 beats/min to 75–90 beats/min (84.5 % of control). This heart failed completely at approximately 2 1/4 hr after having received the toxin.

Parameters measured are calibrated as follows: (1) Heart rate = beats per min, measured on a fast trace by counting each beat for 1 min; (2) ECG: intervals measured in msec, amplitude in mV; (3) Mean blood pressure = an electrical mean which averages systolic and diastolic pressure differences; (4) Arterial blood pressure = measured as systolic and diastolic pressure on a 0 to 200 mmHg scale; (5) Force of contraction = measured on a Walton-Brodie strain gauge.

and force of contraction throughout the 3- to 5-hr observation period (Fig. 2). In one heart there was an immediate ventricular fibrillation which was reversed by electrical defibrillation. The addition of antitoxin appeared to stabilize this heart preparation against further ventricular fibrillation. Of 4 isolated heart preparations, 2 failed at from 1 to 1.5 hr after toxin treatment with progressive loss of force of contraction and cardiac arrest.

Discussion

The cardiac effects of botulinal toxin are direct; they are not occurring as a response to respiratory distress or paralysis. This conclusion is based on a number of facts: (a) ECG effects can occur before signs of respiratory distress; (b) poisoned animals with obvious respiratory distress can have a normal ECG; (c) at autopsy immediately after death from respiratory paralysis, the heart may continue to beat and has a normal appearance; and (b) the strongest evidence of the independence of cardiac effects from respiratory distress is the occurrence of similar toxic effects on isolated *in vitro* heart preparations.

The reason that the cardiac effects have not been recorded in clinical studies of botulism, is probably that the victim does not seek medical attention early in the poisoning. Since the phenomenon is rapidly reversed, the clinician sees the patient at a time when the cardiac effects are no longer evident and support of respiration is the key to therapeutic success. Assuming the human heart is sensitive, ignorance of sensitivity relative to laboratory animals prevents any realistic guess as to the significance of the phenomenon in human botulism. Dr. V. R. Dowell of the Centers for Disease Control, USPHS, Atlanta, Georgia, tells us that in blood or serum from human victims of botulism rarely is there as many as 20 mouse LD_{50}/ml present with 1 or 2 LD_{50} being more commonly detected.

The cardiac studies of Dickson and Shevky (1923) focused on the vagal nerve as the site of action of the toxin. However, vagus innervation may not be the only action of the toxin, since bradycardia was observed in isolated heart preparations. This finding is supported by our experience with vagotomy in the dog. In the intact animal, poisoning of the vagus nerve would predict the occurrence of tachycardia rather than bradycardia. Rosenblum (1966), in studies of the isolated cat heart, concluded that the toxin affected post-ganglionic parasympathetic nerves, but did not specify where in the heart these susceptible nerves were located.

A number of locations can be considered (Napolitano *et al.*, 1965; Löffelholz, 1981; Löffelholz *et al.*, 1982) as sites of action for the toxin: the junction of the vagal nerve with the sino-atrial (SA) node; site of SA-atrioventricular (AV) interaction; His bundle, Purkinje fibers and extranodal cardiac fibers. Our findings with the vagotomized and

atropinized animal and the isolated heart rule against the vagal nerve-SA junction as a primary site of action for the heart changes observed in our study.

Heart muscle cells isolated from the nervous system have an intrinsic capacity to beat. This beat did not change when toxin was added to rat ventricular muscle tissue culture, nor was the viability of the tissue culture cells affected. This finding is consistent with the lack of effect by the toxin in chick heart cell tissue cultures (Lamanna, unpublished observations, 1951).

A remarkable feature of the cardiac effects of the toxin is its ready reversibility. This is in contrast to the experience with other model nerve systems such as the commonly used phrenic nerve-diaphragm muscle *in vitro* preparation. Table IV illustrates this phenomenon of reversibility for both the hemagglutinin-containing toxin and the hemagglutinin-free toxin in the isolated rat heart. There was a striking difference between the intact and isolated heart in the reversibility of cardiac changes following exposure to toxin. In the whole animal, heart changes were reversible, even though exposure to toxin was continued. However, in the isolated rat heart, recovery occurred only after the toxin was washed out of the preparation. The *in vivo* heart, unlike the isolated heart, apparently possesses an adaptive means for reversing cardiotoxicity even with continued exposure to toxin (Tables II and V).

Reversibility of the bradycardia caused by botulinal toxin presumably did not occur in the isolated cat heart since it was not mentioned (Rosenblum, 1966). Vincenzi (1967) reported that, in the rabbit, the beat of the isolated SA-node upon stimulation was irreversibly and completely blocked by type E toxin culture supernatant. However, impure toxin in culture fluid was used in those studies and therefore the findings cannot be definitively compared with our results. In addition, the isolated hearts were not stimulated in our work, so the response measured was completely spontaneous.

Botulinal toxin is known to act on the cholinergic parasympathetic system (Thesleff, 1981). Actions of the toxin may be limited to inhibition of cholinergic nerves, including those in the heart. Because of the ready reversibility, the cardiac effects described in this paper may be a tool of unique value in studies of the sites and mechanisms of action of botulinal toxins. Further investigations of this heart poisoning may yield information about the nature of cardiac innervation that has not as yet been clearly recognized. Among the possibilities for future study is the determination of the absence or presence of linkages of parasympathetic synapses with adrenergic pathways in the heart (Löffelholz, 1981; Löffelholz *et al.*, 1982).

What is the significance of the reversibility of bradycardia and ECG changes for hypotheses of the mechanism of action of botulinal toxin? Reversibility favors, although it does not prove, a physical rather than a

chemical lesion at the site of poisoning by the toxin. For the case of a chemical cause of toxicity, the receptor or site of action of the toxin would be expected to undergo a change in structure with a concomitant loss in the capability to react anew with toxin molecules. Otherwise, for repeated and rapid uptake of toxin (Table IV) at a given specific receptor site, there must be an extraordinary rate of repair of chemical damages at sites of uptake or action of toxin. On the other hand, a physical mode of action, such as an adsorption-desorption phenomenon, would not require time-consuming and repeated in-place synthesis of receptor or other site of action damaged by toxin. Thus, the findings fit a physical mode of action, such as that in the "pipe and valve" hypothesis (Lamanna, 1976; Hanig and Lamanna, 1979; Simpson, 1981).

There may be 2 kinds of chemically different receptors, one in the heart which loosely binds toxin, and another elsewhere in the parasympathetic system which strongly binds toxin. Another possibility is the existence of a chemically identical receptor which differs in the site and manner of its placement in unlike tissues, with consequent variation in the accessibility or penetration of the toxin to the receptor. Either possibility can be accommodated by a purely physical mode of action.

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